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Spore Morphology of Cyatheaceae in China

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Scanning electron microscopy and light microscopy were used in a palynological study of eight species of Cyatheaceae in China: *Sphaeropteris brunoniana*, *Alsophila spinulosa*, *A. latebrosa*, *A. costularis*, *A. denticulata*, *A. gigantea*, *A. austroyunnanensis* and *A. khasyana*. The first three species of *Alsophila* are in subgenus *Alsophila* and the last four are in subgenus *Gymnosphaera*. The spores of nearly all species of *Alsophila* examined are characterized by a ridged or modified ridged perine and a granular or verrucate exine, except the spores of *A. denticulata* and *A. austroyunnanensis*, which have a smooth exine. Species with a granular or verrucate exine may be advanced in comparison with the species with a smooth exine, implying that the species of Cyatheaceae in China are advanced. The spores of *Sphaeropteris brunoniana* feature an incipient granular outermost layer and a verrucate exine. Species of *Alsophila* subgenus *Gymnosphaera* resemble species of subgenus *Alsophila* in having a granular or verrucate exine and a ridged perine, supporting the placement of *Gymnosphaera* within *Alsophila* rather than recognizing it as a separate genus. Because spores in the species of subgenus *Gymnosphaera* are more diverse and have a more complicated perine, it is possible that this group is advanced when compared with other species of *Alsophila*.

Key words: *Alsophila*, China, Cyatheaceae, *Gymnosphaera*, *Sphaeropteris*, spore morphology

Cyatheaceae Kaulf. are a pantropical family of approximate 500-600 species with tree-like trunks. Since the family was established in 1827, considerable attention has been paid to its morphology, anatomy, taxonomy and systematic position (Holttum 1963, 1965, Holttum & Edwards 1983, Holttum & Sen 1961, Tryon 1970, Tryon & Tryon 1982, Lellinger 1987, Lucansky 1974a, b, Lucansky & White 1974, Conant *et al.* 1994, 1995). Still, however, there is no consensus on its delimitation, subdivisions and phylogeny. Holttum & Sen (1961), Tryon (1970) and Lellinger (1987) divided the Cyatheaceae *s. str.* into one, six and four genera, respectively. Ching (1978) classified the 14 species and two varieties of Cyatheaceae in China into three genera; *Sphaeropteris*, *Alsophila* and *Gymnosphaera*, while Xia (1989) placed them in two genera, *Sphaeropteris* and *Alsophila*. The genus *Sphaeropteris* is distinguished from other Cyatheaceae by its uniform stipe scales. It has a pantropical distribution, but is absent from Africa and Madagascar (Large & Braggins 2004). Holttum & Edwards (1983) assumed that *Sphaeropteris* arose in Southeast Asia, its center of distribution. There are two species of *Sphaeropteris* in China; *S. lepifera* and *S. brunoniana*. *Alsophila* has a wide distribution, with its center in the Malesian region. Tryon (1970), Ching (1978), Tryon and Tryon (1982), Lellinger (1987) and Xia (1989) recognized the genus, while Holttum & Edwards (1983) treated it as a section in *Cyathea* subgenus *Cyathea*. Twelve species and two varieties of *Alsophila* are in China. *Gymnosphaera* is a controversial group. Copeland (1947) and Ching (1978) recognized it at the gener-

ic level, while Holttum (1963) treated it as a section of subgenus *Cyathea*, and Xia (1989) as a subgenus of *Alsophila*. Although based on the same series of morphological and anatomical characters, those classification systems are still controversial due to varied taxonomic opinions. The classification we use is according to Xia's two genus system.

In order to revalue the relationship of *Sphaeropteris*, *Alsophila* and *Gymnosphaera*, resorting to the scanning electron microscopy (SEM) and light microscopy, we observed spore morphology of eight species of Cyatheaceae in China, of which one in the genus *Sphaeropteris*; *S. brunoniana*, others in the genus *Alsophila*; *A. spinulosa*, *A. latebrosa*, *A. costularis*, *A. denticulata*, *A. gigantea*, *A. austroyunnannesis* and *A. khasyana* (Table 1). The last four species of *Alsophila* belong to the subgenus *Gymnosphaera*.

Gastony (1974, 1979) and Gastony & Tryon (1976) have done a lot of researches on the spore morphology of Cyatheaceae, but they seldom focused on the Asian species of Cyatheaceae. However, the percentage of endemism of this family in Asia is very high, for example, the species endemism of the family attains 90% in Southeast Asia (excl. Malay Peninsula) (Tryon & Gastony 1975). So it is necessary to add new spore morphology data from species of this region.

Materials and Methods

The sources of the samples used in this study are listed in Table 1. All were obtained from wild plants in August, 2005, except *Alsophila spinulosa* was from a living plant transplanted to the fern garden of the Kunming Institute of Botany, Chinese Academy of Sciences (KIB) and *A. denticulata* was from a herbarium specimen at KUN. Voucher specimens are deposited in KUN.

The spore samples were mounted on aluminum stubs with double sided adhesive tape, coated with a thin layer of gold-palladium, then examined and photographed with a KYKY-AMRAY 1000B Electron Microscope at the Electron Microscopy Center, KIB. The SEM was operated at 25 KV. In all species in which the exine appears to be concealed by a perine layer during any stage of spore development, the spores were boiled sufficiently long (examined intermittently by light microscope) in 1 N hot sodium hydroxide to remove the perine, as described by Gastony (1974). These alkali-prepared spores were then washed with distilled water, suspended in a few drops of 70% ethanol, and piped to double sided adhesive tape attached to stubs for SEM study. Micrographs of the proximal and/or distal faces and of some ornamentation details were photographed to compare spore morphology at each orientation.

Results

The spores of all species of Cyatheaceae investigated were trilete, radially symmetrical, heteropolar and perinous (Figs. 1-24). The polar outline is triangular, usually with concave sides and rounded angles. In equatorial view, the proximal face appears as a low pyramid, while the distal face is hemispherical to convex.

Of the two species of *Sphaeropteris* in China, only *S. brunoniana* was accessible for SEM observation. The spores of *S. brunoniana* are perinous with a verrucate exine (Fig. 1). Figure 2 shows an alkali-untreated spore with a densely granular outermost layer.

The spores of *Alsophila spinulosa* feature a ridged perine and a verrucate exine (Figs. 3-4). The laesurae are wide, slightly elevated, are distinct under the coarse perine and each one is grooved (Fig. 3). The ridges more or less connect with each other. Some granules are scattered on the surface of the perine. Figure 5 shows a spore after being treated with sodium hydroxide to demonstrate the differential expansion and perinous nature of the outermost layer.

TABLE 1. Specimens of Cyatheaceae examined by scanning electron microscopy and light microscopy and the results.

Taxon	Locality and altitude	Voucher in KUN	Perine surface	Exine surface	Figure no.
Sphaeropteris brunoniana (Hook.) R. M. Tryon	Xiaola highway, Mengla, Xishuangbanna, 1050m	*X.Cheng 101*	pointed-projections	verrucate	1-2
Alsophila spinulosa (Wall. ex Hook.) R. M. Tryon	Transplanted plant in KIB, 1928m	*X. Cheng 103*	ridges	verrucate	3-5
A. latebrosa Wall. ex Hook.	Daweishan, HeKou, 900m	*X. Cheng 106*	ridges	granular	6-8
A. costularis Baker	Adeboxiang, Jinping, 1500m	*X. Cheng 102*	ridges	verrucate	9-10
A. denticulata Baker	Caoguoshan, Xichou, 1520m	*No. 0006272*	ridges with rods and echinates	smooth	11-14
A. gigantea Wall. ex Hook.	Xiaola highway, Mengla, Xishuangbanna, 1050m	*X. Cheng 100*	fused strands	granular	15-18
A. austroyunnanensis S. G. Lu	Hekou 3 km point way to Pingbian, 1460m	*X. Cheng 108*	ridges with echinates	smooth	19-21
A. khasyana T. Moore ex Kuhn	Hekou 3 km point way to Pingbian, 1460m	*X. Cheng 109*	ridges	verrucate	22-24

The spores of *Alsophila latebrosa* are characterized by a typical ridged perine (Figs. 6-7) and a granular exine (Fig. 8). The laesurae are covered by a perine and are obscure. The elongated and branched ridges are fewer on the proximal surface than on the distal surface. Granules are scattered on both the ridges and on the surface of the perine.

The spores of *Alsophila costularis* also share the typical ridged perine (Fig. 9), but the ridges are shorter and lower than those on the spores of *A. latebrosa*. Some granules are scattered on the perine surface. The exine is verrucate, even on the laesurae regions (Fig. 10).

Alsophila denticulata, *A. gigantea*, *A. austroyunnanensis* and *A. khasyana* are here placed in subgenus *Gymnosphaera*. The spores of *A. denticulata* also have a ridged perine, with the ridges somewhat connected to each other (Figs. 11-12), but there are some rod-shaped appendages (Fig. 11) or echinate or pointed projections (Fig. 12) on the ridges. Some granules are scattered on the ridges and on the rest of the surface of the perine. The laesurae are concealed by the perine and could not be observed. Figure 13 shows the exine of a spore of *A. denticulata* prepared in sodium hydroxide. The exine is nearly smooth, except for a few scattered protuberances, and the laesurae are distorted slightly inward.

Alsophila gigantea represents a different kind of spore morphology (Figs. 15-18). Dense woven or fused strands constitute the perine surface and even the laesurae regions and show no difference between the proximal and distal surfaces. Narrow laesurae are conspicuous. The exine also has granules (Fig. 18) like those of *A. latebrosa* (Fig. 8).

Alsophila austroyunnanensis, endemic to China, is characterized by its dimorphic fronds. Because of its echinate stipes, this species was included in subgenus *Alsophila* (Lu 1998). Based on molecular data, *A. austroyunnanensis* is placed in subgenus *Gymnosphaera* (Li *et al.* 2004, Lu & Li 2005). The perine of its spores has short ridges,

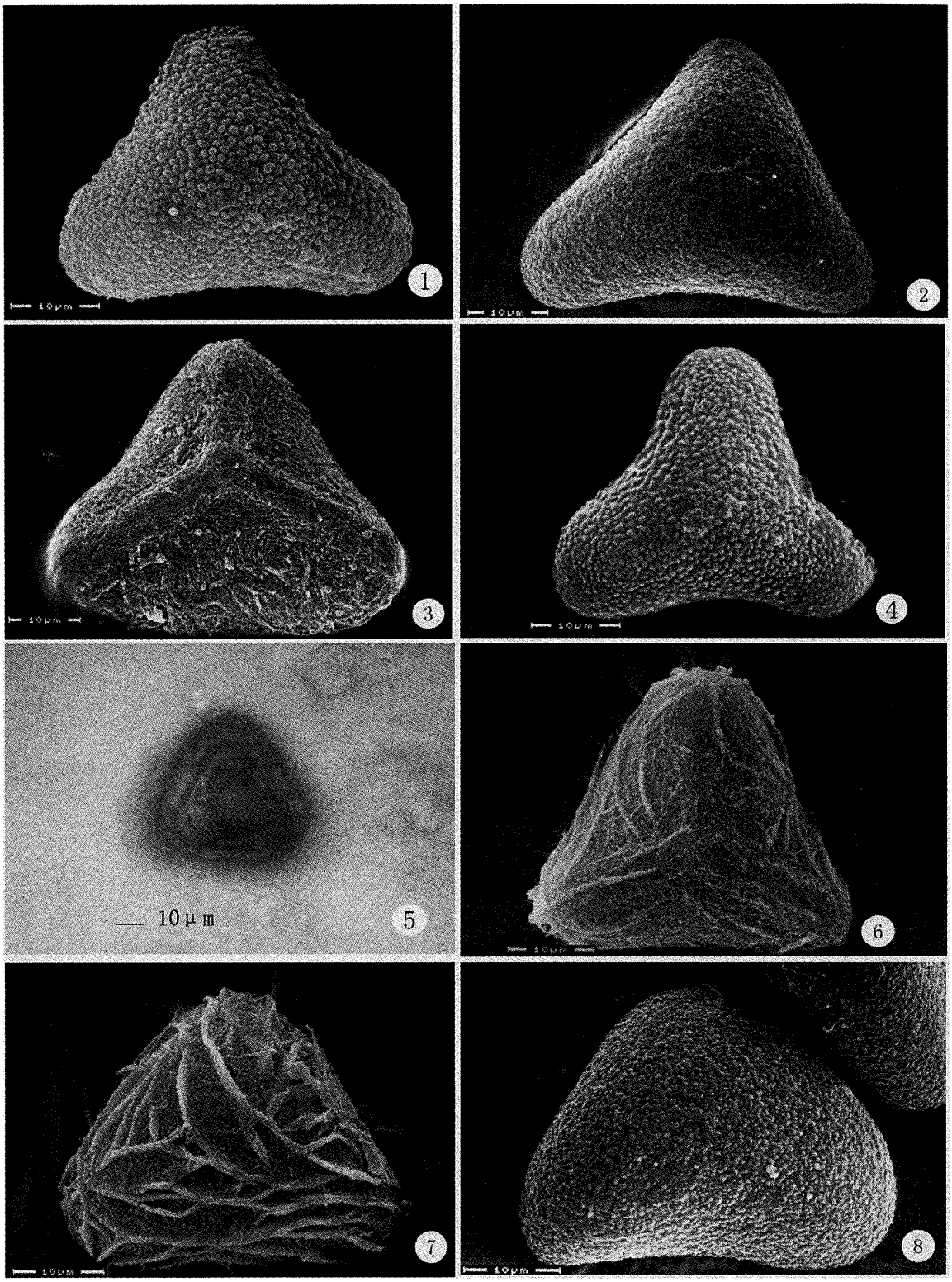
which are nearly absent in the laesurae regions (Fig. 19). The laesurae are wide and grooved. The ridges are somewhat connected with each other and have echinate projections (Fig. 20). Figure 21 shows a spore with a nearly smooth exine. Perhaps the spore was treated insufficiently with alkali; there are some low verrucae and perine remaining on the surface of the exine. This pattern differs from that in the species of *Alsophila* with granular exine. The spores of *A. austroyunnanensis*, however, share the smooth exine type as described by Gastony & Tryon (1976) for the spores of all neotropical and most paleotropical species of *Alsophila*. The echinate projections on perine ridges, however, recall some paleotropical species of *Sphaeropteris* with similar perine morphology (Gastony & Tryon 1976).

The spores of *A. khasyana* are also very special among the species of Cyatheaceae in China. The spores represent a coarse perine with short, irregularly deposited ridges (Figs. 22-23). The exine is verrucate (Fig. 24). The wide laesurae are also obvious.

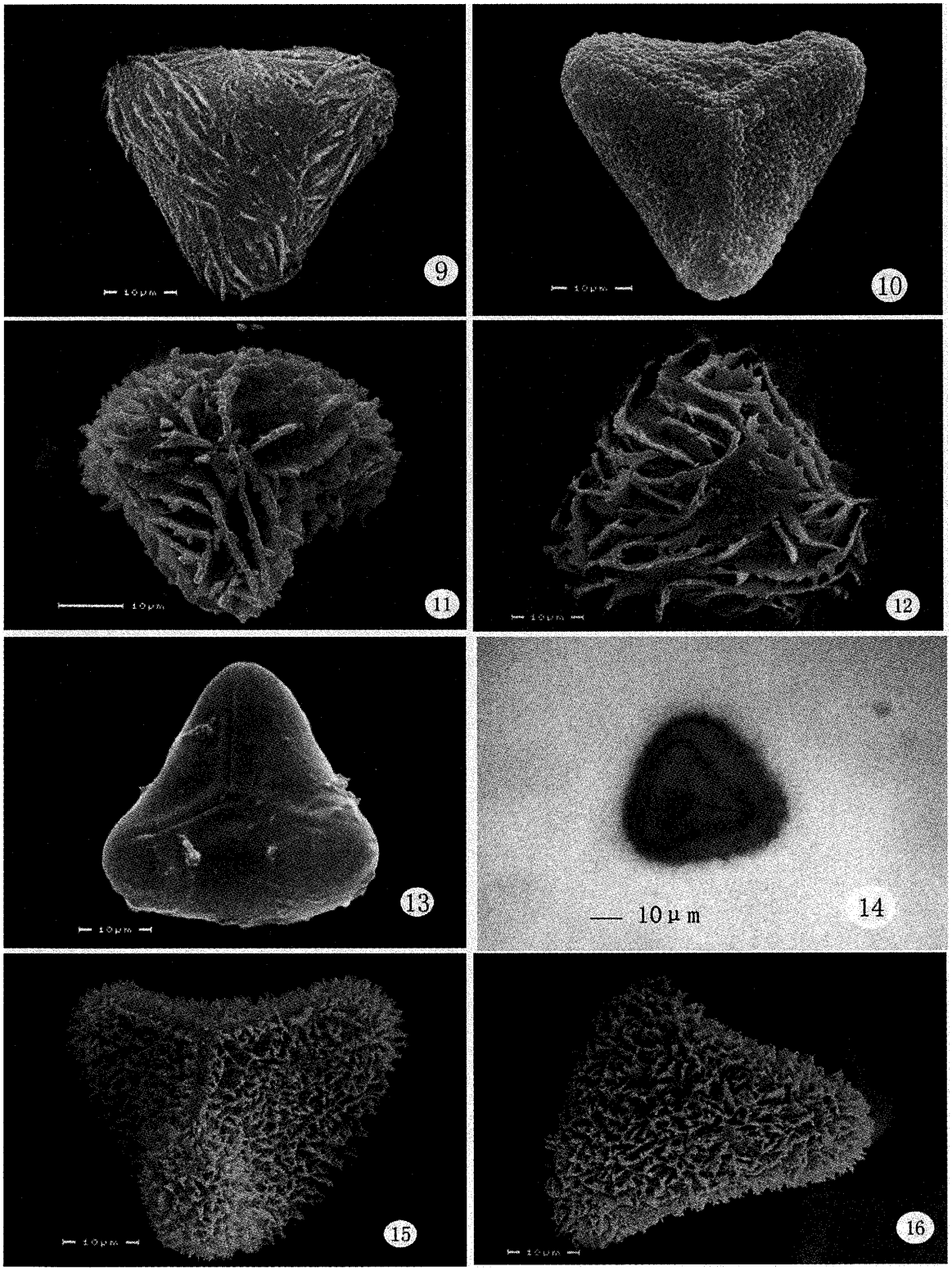
Discussion

Gastony & Tryon (1976) reported the perine of the spores of the paleotropical species of *Sphaeropteris* to have pointed projections, and usually smooth exine without sculpturing. The spores of the neotropical species, excluding those in the *S. horrida* group, have hair-like or sometimes incipient granular (*S. elongata*) perine and pitted or verrucate exine. They also reported the spores of *Alsophila* species to be characterized by a typical type of ridged perine and smooth exine. In the present study, the species were not all the same. *Sphaeropteris brunoniana* had a granular outermost layer and a verrucate exine. Gastony & Tryon (1976) reported the presence of granules external to the exine in certain collection of some species of *Sphaeropteris* such as *S. elongata* (p. 744, Fig. 38 and 39) to be an incipient stage in perine deposition occurring late in sporogenesis. Cao *et al.* (2007, p. 12, Figs. 21-24) reported that *S. brunoniana* spores develop a short echinulate perine. The spores with shortly echinulate perine may be comparable to those with spaced pointed projections, as Gastony & Tryon (1976) described in the perine of all paleotropical species of *Sphaeropteris* and for the neotropical *S. horrida* group. *Sphaeropteris brunoniana* possibly developed a perine with pointed projections, but additional information on spore wall development and a molecular phylogenetic analysis is needed to further clarify the systematic position of this species.

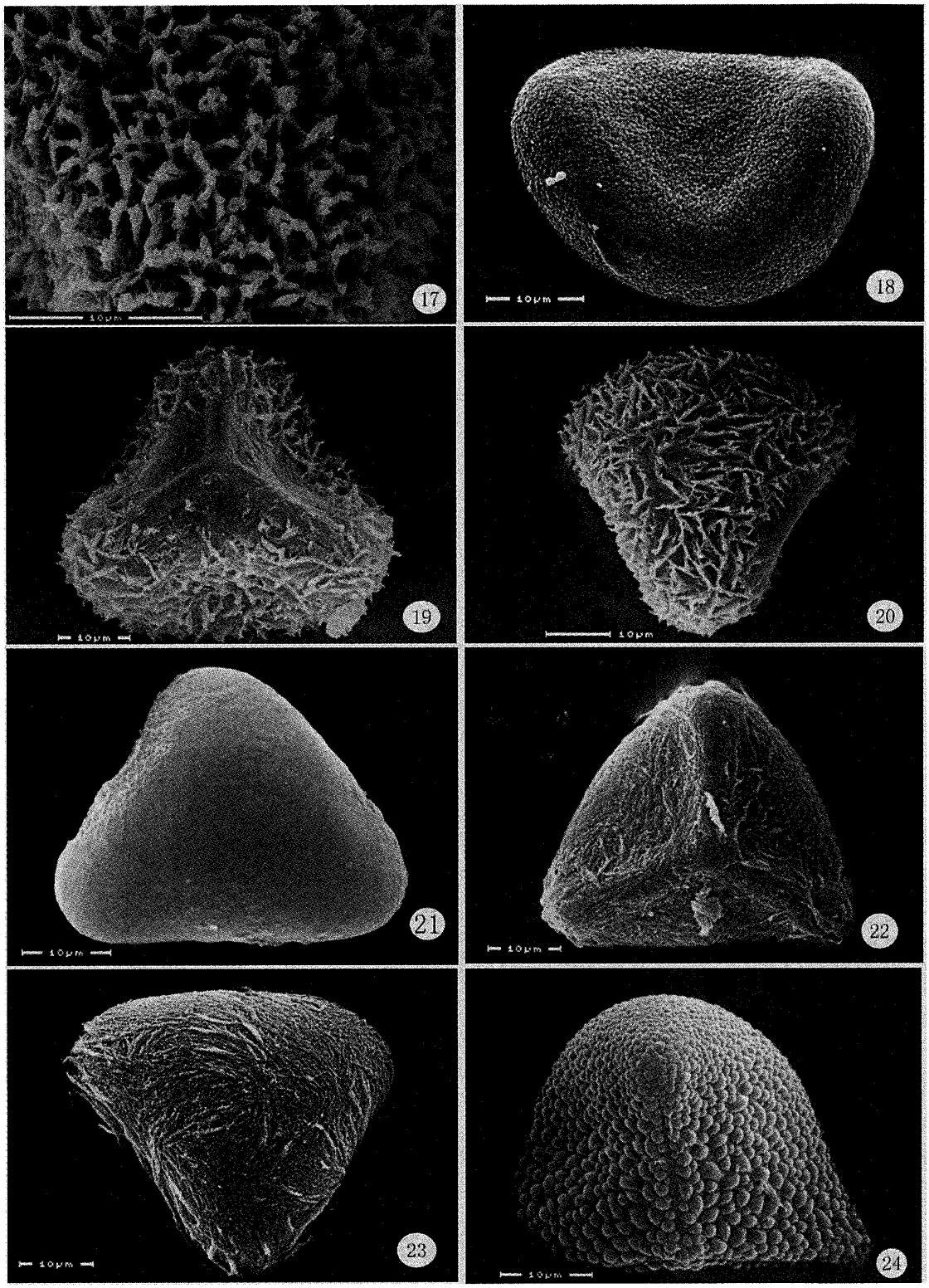
Most spores of the species of *Alsophila* examined feature a ridged or modified ridged perine and a granular or verrucate exine, except that the spores of *A. denticulata* and *A. austroyunnanensis* develop a type of smooth exine. The exine surface of the spores of *A. latebrosa* and *A. gigantea* bear granules. The spores of *A. spinulosa*, *A. costularis* and *A. khasyana* with verrucate exine recall the spore type of *S. brunoniana*. The spores of subgenus *Gymnosphaera* resemble those of other species of *Alsophila* in having ridged perine, suggesting that *Gymnosphaera* is closer to *Alsophila* than to *Sphaeropteris* based on spore morphology. At this point, it supports the traditional classification that *Gymnosphaera* is attached to *Alsophila* (Holttum 1963, Tryon & Tryon 1982, Xia 1989). Apart from this, the species of *Gymnosphaera* have diverse spore characters. Their perine morphologies are more complicated than those of other species of *Alsophila*, and some perine appendages are similar to those of many paleotropical species of *Sphaeropteris* (Gastony & Tryon 1976), implying that *Gymnosphaera* is either an advanced group derived from the *Alsophila* group, with spores characterized by the simpler typical ridged perine, or is an intermediate group between *Sphaeropteris* and other species of *Alsophila* in spore evolution and conservation of the rod-like and pointed appendages on the ridges of the perine.



FIGS. 1-8. Perine and exine morphology characteristics. — 1-2. *Sphaeropteris brunoniana*: 1. Distal face of spores after treated in sodium hydroxide showing verrucate exine; 2. Distal face of untreated spores showing incipient granular perine disposition over the exine. — 3-5. *Alsophila spinulosa*: 3. Proximal face showing coarse perine surface; 4. Distal face showing verrucate exine of a spore after chemical removal of perine; 5. Light micrograph of spore after preparation with sodium hydroxide to demonstrate differential expansion and perinous nature of the outermost layer. — 6-8. *A. latebrosa*: 6. Proximal face; 7. Distal face showing perine with ridges and granules; 8. Distal face showing granulated exine before perine disposition.



FIGS. 9-16. Perine and exine morphology characteristics. — 9-10. *A. costularis*: 9. Distal face showing ridged perine; 10. Proximal face showing verrucate exine after chemical removal of perine. — 11-14. *A. denticulata*: 11. Proximal face showing rod appendages on perine ridges; 12. Distal face showing echinate projections on perine ridges; 13. Proximal face showing smooth exine after chemical removal of perine; 14. Light micrograph of spore after alkali-treatment to demonstrate differential expansion and perinous nature of the outermost layer. — 15-16. *A. gigantea*: 15. Proximal face showing perine; 16. Distal face showing perine.



FIGS. 17-24. Perine and exine morphology characteristics. — 17-18. *A. gigantea*: 17. High magnification of perine surface; 18. Lateral side showing granulated exine. — 19-21. *A. austroyunnanensis*: 19. Proximal face showing fewer ridges of perine on the laesurae regions; 20. Distal face showing ridged perine with echinate projections; 21. Distal face showing nearly smooth exine after chemical removal of perine. — 22-24. *A. khasyana*: 22. Proximal face showing perine; 23. Distal face showing perine; 24. The round angle showing verrucate exine.

As to the inter-/intra-generic relationship in Cyatheaceae, there are several different opinions. Holttum and his colleagues suggested that the *Alsophila* and *Cyathea* clades are primitive, based on features of the sori and indusia, and the *Sphaeropteris* clade without an indusium is derived (Holttum & Sen 1961, Holttum & Edwards 1983). In contrast, Tryon and Tryon (1982) proposed that *Sphaeropteris* without indusium is primitive, while *Alsophila* and *Cyathea* are derived. According to the hypothesis that the indusia of Cyatheaceae are homologous with those of Dicksoniaceae and that the indusiate group is primitive (Holttum & Sen 1961), it can be postulated that *Gymnosphaera* without an indusium is advanced within the genus *Alsophila*. According to cpDNA restriction site mutations, Conant *et al.* (1994, 1995) and Stein *et al.* (1997) proposed three major evolutionary lineages within the Cyatheaceae; the *Alsophila* clade, the *Cyathea* clade and the *Sphaeropteris* clade. They speculated that the *Alsophila* clade is the most basal clade, while the *Cyathea* and *Sphaeropteris* clades are derived sister groups. Wang *et al.* (2003), based on chloroplast *trnL* intron and *trnL-F* intergeneric spacer sequences of the species of Cyatheaceae in China, proposed that *Sphaeropteris* is the basal group, while *Alsophila* and *Gymnosphaera* are sister groups derived from sphaeropteroid ancestors. In our investigation, the results suggest that *Gymnosphaera* has a closer relationship with *Alsophila* than with *Sphaeropteris* and support Xia's subdivision; while the relationships between *Sphaeropteris* and *Alsophila* and *Sphaeropteris* and *Gymnosphaera* are ambiguous. It is therefore still necessary to seek new data to clarify their systematic position. It can be added that the granular or verrucate exine of Chinese Cyatheaceae, compared to the smooth exine of other paleotropical species, may be an advanced character. This possibility implies that the species of Cyatheaceae in China, comprising the northern distribution of the family, are advanced.

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